09/694,758 #10 Attachment

**PCT** WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/68, G01N 33/68 — A2

(11) International Publication Number: WO 00/14283

(43) International Publication Date: 16 March 2000 (16.03.00)

(21) International Application Number: PCT/US99/20098

(22) International Filing Date: 3 September 1999 (03.09.99)

(30) Priority Data:
09/146,969 4 September 1998 (04.09.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/146,969 (CON)
Filed on 4 September 1998 (04.09.98)

(71) Applicant *(for all designated States except US)*: WASHINGTON UNIVERSITY [US/US]; 660 South Euclid Avenue, Campus Box 8013, St. Louis, MO 63110 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: DIECKGRAEFE, Brian, K. [US/US]; Washington University School of Medicine, Department of Gastroenterology, Box 8124, 660 South Euclid Avenue, St. Louis, MO 63110 (US).

(74) Agents: KAGAN, Sarah, A. et al.; Banner & Witcoff, Ltd., 11th floor, 1001 G Street, N.W., Washington, DC 20001–4597 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**
*Without international search report and to be republished upon receipt of that report.*

(54) Title: GENE MARKERS FOR CHRONIC MUCOSAL INJURY

(57) Abstract

The invention provides gene markers for chronic mucosal injury and ulcerative colitis. Expression products of the *REG* gene family can be used to detect the presence of chronic mucosal injury in a body sample of a human. The expression products of a gene represented by a Hs.111244 polynucleotide can be used to detect ulcerative colitis in a body sample of a human. Further, these markers can be used to differentiate humans with chronic mucosal injury from humans with common acute inflammatory colon disorder, common non–inflammatory benign colon disorder, and healthy colons. The degree of injury to the colon from chronic mucosal injury can be determined and the efficacy of therapy for chronic mucosal injury can be monitored. A method of screening compounds for anti–chronic mucosal injury and anti–ulcerative activity is also provided by these gene markers.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | | | TR | Turkey |
| BG | Bulgaria | HU | Hungary | ML | Mali | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MN | Mongolia | UA | Ukraine |
| BR | Brazil | IL | Israel | MR | Mauritania | UG | Uganda |
| BY | Belarus | IS | Iceland | MW | Malawi | US | United States of America |
| CA | Canada | IT | Italy | MX | Mexico | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NE | Niger | VN | Viet Nam |
| CG | Congo | KE | Kenya | NL | Netherlands | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NO | Norway | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | NZ | New Zealand | | |
| CM | Cameroon | | | PL | Poland | | |
| CN | China | KR | Republic of Korea | PT | Portugal | | |
| CU | Cuba | KZ | Kazakstan | RO | Romania | | |
| CZ | Czech Republic | LC | Saint Lucia | RU | Russian Federation | | |
| DE | Germany | LI | Liechtenstein | SD | Sudan | | |
| DK | Denmark | LK | Sri Lanka | SE | Sweden | | |
| EE | Estonia | LR | Liberia | SG | Singapore | | |

# GENE MARKERS FOR CHRONIC MUCOSAL INJURY

## BACKGROUND OF THE INVENTION

Clinical assessment of disease activity in ulcerative colitis or Crohn's disease is very difficult. Patient symptoms do not necessarily correlate with the inflammatory (disease) activity in the small intestine and colon, leading to educated guesses being used to direct anti-inflammatory therapy. Similar difficulty exists in measuring or testing the efficacy of new therapeutic compounds. Currently the gold standard in diagnosing ulcerative colitis or Crohn's disease is the use of fiberoptic endoscopy coupled with multiple biopsies and pathologic analysis. This very expensive approach requires a skilled specialist and has associated risks, such as risk of sedation, bleeding, and colon perforation. The patient is also subjected to discomfort from the procedure and preparation.

A less invasive and less risky assessment of mucosal disease activity is needed to accurately guide treatment and to provide an objective measure of mucosal injury, both for patients and for use in clinical studies. There is also a need for a simple test to aid in the differentiation of chronic inflammatory disease (UC or CD) from common acute inflammatory disorders or common non-inflammatory benign disorders. There is a further need for a simple method for the differentiation of ulcerative colitis and Crohn's disease because the surgical and

medical management for these two diseases is profoundly different.

## SUMMARY OF THE INVENTION

It is an object of the invention to provide a method for identifying chronic mucosal injury in a human.

It is another object of the invention to provide a method of determining the degree of injury to the small intestine or colon of a human with chronic mucosal injury.

It is yet another object of the invention to provide a method for monitoring the efficacy of therapy for chronic mucosal injury.

It is a further object of the invention to provide a method of screening compounds for anti-chronic mucosal injury or anti-ulcerative colitis activity.

These and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention provides a method of diagnosing chronic inflammatory bowel disease. At least one gene expression product of the *regenerating (REG)* gene family is detected in a body sample of a human who is suspected of having chronic inflammatory bowel disease. The human is identified as having chronic inflammatory bowel disease if the gene expression product is detected.

A further embodiment of the invention provides a method to aid in the differentiation of chronic mucosal injury from common acute inflammatory colon disorder and common non-inflammatory benign colon disorder in a human with symptoms of bowel disease. The amount of at least one gene expression product of the *REG* gene family in a body sample of a first human who is suspected of having bowel disease, is compared with the amount of the gene expression

product in a body sample of a second human who is healthy. The first human is identified as having chronic mucosal injury if the body sample of the first human contains more of the gene expression product than the body sample of the second human.

Another embodiment of the invention provides a method to determine degree of injury to small intestine or colon tissue of a human with chronic mucosal injury. A quantity of a gene expression product of the *REG* gene family in a body sample of a human having chronic mucosal injury is determined. The amount is correlated with the degree of injury to the small intestine or colon.

Still another embodiment of the invention provides a method of monitoring the efficacy of therapy for chronic mucosal injury in a human body sample. At least one gene expression product of the *REG* gene family is quantitated in a body sample of a human who has been subjected to therapy for chronic mucosal injury. The quantity of the expression product in the sample is compared to the quantity of the gene expression product in a matched body sample of the human at an earlier time. A reduction in the quantity of the gene expression product after therapy is an index of efficacy of the therapy.

Another embodiment of the invention provides a method of screening compounds for anti-chronic mucosal injury activity. A colonic cell expressing a gene which is a member of the *REG* gene family is contacted with a test compound. The expression of the *REG* gene is quantitated. A test compound which decreases expression of the gene is identified as a potential compound for treating chronic mucosal injury.

A further embodiment of the invention provides a method of diagnosing ulcerative colitis. An mRNA which is expressed by a gene represented by a Hs.111244 polynucleotide is detected in

a body sample of a first human who is suspected of having ulcerative colitis. The human is identified as having ulcerative colitis if the mRNA is detected.

Still another embodiment of the invention provides a method to aid in the differentiation of ulcerative colitis from common acute inflammatory colon disorder, common non-inflammatory benign colon disorder, and Crohn's disease in a human with symptoms of bowel disease. The amount of mRNA which is expressed by a gene represented by a Hs.111244 polynucleotide in a body sample of a first human suspected of having bowel disease is compared with the amount of the mRNA in a comparable body sample of a second human who is healthy. A body sample of the first human which contains more of the mRNA than the body sample of the second human identifies the first human as having ulcerative colitis.

Another embodiment of the invention provides a method to determine the degree of injury to small intestine or colon tissue of a human with ulcerative colitis. A quantity of an mRNA which is expressed by a gene represented by a Hs.111244 polynucleotide in a body sample of a first human having ulcerative colitis is determined. The quantity of the mRNA is correlated with the degree of injury to the small intestine or colon.

Even another embodiment of the invention provides a method of monitoring the efficacy of therapy for ulcerative colitis in a human body sample. An mRNA which is expressed by a gene represented by a Hs.111244 polynucleotide is quantitated in a body sample of a human who has been subjected to therapy for ulcerative colitis. The quantity of the mRNA in the sample is compared to the quantity of the mRNA in a matched body sample of the human at an earlier time. A reduction in the quantity of the mRNA after therapy is an index of efficacy of the therapy.

Still another embodiment of the invention provides a method of screening compounds for

anti-ulcerative colitis activity. A colonic cell expressing an mRNA which is expressed by a gene represented by a Hs.111244 polynucleotide is contacted with a test compound. The expression of the mRNA by the cell is quantitated. A test compound which decreases expression of the mRNA is identified as a potential compound for treating ulcerative colitis.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 demonstrates the hybridization signal of spiked controls. The fluorescence intensity for different levels of gene expression was standardized by spiking a known amount of control genes.

Figure 2 demonstrates the expression of *PSP*, *PAP*, and *REGH* in inflammatory bowel disease.

Figure 3 demonstrates the use of reverse transcriptase PCR with primers specific for *PAP*, *PSP*, or *REGH* on mRNA isolated from a healthy human and a human with ulcerative colitis.

Figure 4 demonstrates the purification of recombinant PSP and REGH proteins.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The inventor has discovered that chronic mucosal injury can be diagnosed by detecting expression levels of the *REG* gene family and a gene represented by a Hs.111244 polynucleotide in a human body sample. The members of the *REG* gene family have been found to be strongly expressed in regions of the colon involved with chronic mucosal injury and in the small intestine and colon of humans with Crohn's disease. Additionally, a gene represented by the Hs.111244 polynucleotide is strongly expressed in the colon of humans with ulcerative colitis. Such strong

expression is both surprising and useful because the *REG* gene family and the gene represented by the Hs.111244 polynucleotide are not expressed, or are expressed at low levels, in healthy small intestine and colon tissue. Further, the expression products of the *REG* gene family and the gene represented by the Hs.111244 polynucleotide can be detected in the serum of humans with chronic mucosal injury and ulcerative colitis, respectively.

Chronic mucosal injury can be caused by inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. Crohn's disease affects both the small intestine and the colon. Chronic mucosal injury can further be caused by immunodeficiencies, such as chronic granulamatous disease and transplantation rejection, and infections, such as mycobacteria.

At the present time, the human regenerating (*REG*) gene family is known to contain four genes: *pancreatic stone protein (PSP)* (the protein is also known as thread protein, lithostathine, and Reg) as shown in SEQ ID NO:1, *pancreatitis-associated protein (PAP)* as shown in SEQ ID NO:2, *human pancreatic beta cell growth factor*, also known as *(INGAP)*, as shown in SEQ ID NO:3, and *regenerating gene homologue (REGH)* as shown in SEQ ID NO:4. In normal circumstances, these genes are regionally expressed in low amounts in the small bowel and pancreatic epithelium. Healthy colonic mucosa and small intestine has little or no expression of the *REG* gene family. Ulcerative colitis, Crohn's disease, or other chronic mucosal injury leads to high levels of *REG* gene expression in the colonic mucosal or small intestine or both. This expression of the members of the *REG* gene family correlates with the degree of histopathological injury, and is not seen in the setting of acute self-limited colonic inflammation or common non-inflammatory benign colon disorders.

The nucleic acid sequence of the expressed sequence tag (EST) Hs.111244 is shown in

SEQ ID NO:5. The nucleic acid sequence of Hs.111244 has been newly determined and represents a more complete sequence of Hs.111244 than has been previously published. This nucleic acid sequence is referred to herein as the Hs.111244 polynucleotide. The gene represented by the expressed sequence tag (EST) Hs.111244, is not expressed or is expressed at low levels in the healthy colon mucosa, acute self-limited colonic inflammation or common non-inflammatory benign colon disorders, or mucosa affected by Crohn's disease. However, the gene represented by the Hs.111244 polynucleotide is expressed at high levels in colonic mucosa affected by ulcerative colitis.

In humans who have been diagnosed with a bowel disease, detection of levels of at least one gene expression product of the *REG* gene family in a body sample can be used to diagnose or prognose chronic mucosal injury or to monitor treatment of chronic mucosal injury. The body sample is obtained from a human and can be, for example, a tumor, a solid tissue such as colon or small intestine tissue, or a fluid sample such as blood, serum, or plasma. The human from whom the body sample is obtained can be apparently healthy or can already be identified as having chronic mucosal injury. A comparable body sample is a body sample obtained from a second human which is the same type of body sample as obtained from a first human. A matched body sample is a body sample obtained from the first human at an earlier time which is the same type of body sample from the first human obtained at a later time.

Expression products of the *REG* gene family can be detected in a body sample. Detection of the expression products in a human's body sample indicates the presence of chronic mucosal injury in the human. In one embodiment, the body sample is assayed for the presence of at least one REG gene family protein. A REG gene family protein or polypeptide, can be detected using,

for example, anti-REG gene family-specific antibodies. The antibodies can be labeled, for example, with a radioactive, fluorescent, biotinylated, or enzymatic tag and detected directly, or can be detected using indirect immunochemical methods, using a labeled secondary antibody. The presence of REG gene family protein or polypeptides can be assayed, for example, in tissue sections by immunocytochemistry, or in lysates, using Western blotting, as is known in the art. Further, REG gene family proteins or polypeptides can be assayed by immunoprecipitation assay, enzyme-linked immunoabsorbant assay, quantitative antigen capture-based immunoassay, and radioimmunoassay.

The level of at least one REG gene family protein or polypeptide in a body sample of a human suspected of having a chronic mucosal injury can be compared with the level of the protein or polypeptide in a healthy body sample. The level of a REG gene family protein or polypeptide in a body sample of a human suspected of having chronic mucosal injury can be determined using antibodies specific for the REG gene family protein or polypeptide. The level of the REG gene family protein or polypeptide in a healthy body sample can also be determined. The two levels are compared to each other and a higher level of the REG gene family protein or polypeptide in the suspect human's body sample as compared to the healthy human's body sample indicates the presence of chronic mucosal injury in the suspect human. Preferably, the increased level of the REG gene family protein in the suspect sample is at least 25%, 50%, 100%, 150%, 200% or 250% higher than in the healthy body sample.

Alternatively, the presence of mRNA expressed from at least one member of the *REG* gene family or mRNA expressed from the gene represented by the Hs.111244 polynucleotide can be detected in a body sample. Detection of mRNA expressed from at least one member of the

*REG* gene family in a body sample of a human indicates the presence of chronic mucosal injury in the human. Detection of mRNA which is expressed by a Hs.111244 polynucleotide in a body sample of a human suspected of having bowel disease indicates the presence of ulcerative colitis in the human.

mRNA expressed from the *REG* gene family or the gene represented by a Hs.111244 polynucleotide can be detected by any means know in the art. For example, one can use ***in situ*** hybridization in tissue sections or Northern blots containing poly $A^+$ mRNA. Other techniques such as high density DNA array hybridization, ribonuclease protection assay, and serial analysis of gene expression can also be used. *REG* gene family- or Hs.111244-specific oligonucleotide probes can be generated using the polynucleotide sequences of the *REG* gene family or of the gene represented by a Hs.111244 polynucleotide. The probes are preferably at least 12, 14, 16, 18, 20, 22, 24, or 25 nucleotides in length and can be less than 2, 1, 0.5, 0.1, or 0.05 kb in length. The probes, for example, can be synthesized chemically, generated from longer polynucleotides using restriction enzymes, or amplified enzymatically. The probes can be labeled, for example, with a radioactive, biotinylated, or fluorescent tag. A mixture of probes can also be used. Such mixture can contain a plurality of probes which are specific to different *REG* family genes or specific for the gene represented by the Hs.111244 polynucleotide. Alternatively, each of a plurality of probes can be used separately.

One of skill in the art can readily determine differences in the amount of *REG* gene family mRNA or a gene represented by a Hs.111244 polynucleotide mRNA transcripts between two body samples, for example, using Northern blots and nucleotide probes. The level of mRNA expressed from of at least one member of the *REG* gene family or the gene represented by the

Hs.111244 polynucleotide in a body sample of a human suspected of having chronic mucosal injury, can be compared with the mRNA expression from at least one member of the *REG* gene family or the gene represented by Hs.111244 polynucleotide in a healthy body sample. This can be done, for example, using *in situ* hybridization in tissue section or in Northern blots containing poly $A^+$ mRNA. A higher level of mRNA expressed from a gene represented by a Hs.111244 polynucleotide in the suspect body sample as compared to the healthy body sample is indicative of ulcerative colitis in the suspect human who has provided the body sample. A higher level of mRNA expressed from a *REG* family gene in the suspect body sample as compared to the healthy body sample is indicative of chronic mucosal injury in the suspect human who has provided the body sample. Preferably, the increased level of mRNA expressed from a member of the *REG* gene family or the gene represented by the Hs.111244 polynucleotide in the suspect body sample is at least 25%, 50%, 100%, 150%, 200%, or 250% higher than in the healthy body sample.

If desired, the level of a particular mRNA, polypeptide, or protein expressed from a *REG* gene family member or mRNA expressed from a gene represented by a Hs.111244 polynucleotide in a body sample can be quantitated. Quantitation can be accomplished, for example, by comparing the level of expression product detected in the body sample with the level of expression product present in a standard curve. A comparison can be made visually or using a technique such as densitometry, with or without computerized assistance.

In a preferred embodiment, chronic mucosal injury can be differentiated from common acute inflammatory colon disorder and common non-inflammatory benign colon disorder in a human with symptoms of bowel disease. The amount of at least one gene expression product such as mRNA or protein of the *REG* gene family in the suspect body sample is compared to the

amount of the same gene expression product in a body sample of a human which is healthy. The gene expression products in the two samples can be compared by any means known in the art. A body sample from a human suspected of having bowel disease which contains more of the gene expression product than the body sample of the healthy human identifies the suspect human as having chronic mucosal injury. Preferably the amount of the gene expression product in the body sample of the human with chronic mucosal injury is increased by at least 25%, 50%, 75%, 100%, 200%, or 250%.

Further, ulcerative colitis can be differentiated from common acute inflammatory colon disorder, common non-inflammatory benign colon disorder, and Crohn's disease in a human with symptoms of bowel disease. The amount of mRNA which is expressed by a gene represented by a Hs.111244 polynucleotide in a first body sample of a human suspected of having bowel disease is compared with the amount of the mRNA in a body sample of a second human which is healthy. The amount of mRNA in the two samples can be compared by any means known in the art. A body sample from a human suspected of having bowel disease which contains more of the mRNA than the body sample of the healthy human identifies the suspect human as having ulcerative colitis. Preferably the amount of mRNA in the body sample of the human with ulcerative colitis is increased by at least 25%, 50%, 75%, 100%, 200%, or 250%.

The degree of injury to the small intestine or colon tissue of a human with chronic mucosal injury can be determined by measuring the quantity of a gene expression product, such as mRNA or protein, of the *REG* gene family in a body sample of the human. The quantity of the gene expression product is correlated with the degree of injury to the small intestine or colon.

Further the degree of injury to the colon tissue of a human with ulcerative colitis can be

determined by measuring the quantity of a mRNA which is expressed by a Hs.111244 polynucleotide or the gene represented by it in a body sample of the human. The quantity of the mRNA is correlated with the degree of injury to the colon.

The efficacy of therapy for chronic mucosal injury can be monitored in a body sample of a human with chronic mucosal injury. At least one gene expression product of the *REG* gene family can be quantitated in a body sample of a human which has been subjected to therapy for chronic mucosal injury. The quantity of the gene expression product in a matched body sample is compared to the quantity of the gene expression product in the body sample at an earlier time. A reduction of in the quantity of the gene expression product after therapy is an index of efficacy of the therapy. Preferably, the amount of the gene expression product is decreased by at least 10%, 25%, 50%, 75% or 100%.

The efficacy of therapy for ulcerative colitis in a human body sample can also be monitored. An mRNA which is expressed by a Hs.111244 polynucleotide or the gene represented by it is quantitated in a body sample of a human which has been subjected to therapy for ulcerative colitis. The quantity of the mRNA in the sample is compared to the quantity of the mRNA in the matched body sample at an earlier time. A reduction of in the quantity of the mRNA after therapy is an index of efficacy of the therapy. Preferably, the amount of the mRNA is decreased by at least 10%, 25%, 50%, 75% or 100%.

According to another aspect of the invention, compounds which have anti-chronic mucosal injury or anti-ulcerative colitis activity can be identified. A colonic cell expressing a gene of the *REG* family or a gene represented by the Hs.111244 polynucleotide can be contacted with a test compound. The test compound can be a pharmacologic agent already known in the art or can

be a compound previously unknown to have any pharmacological activity. The test compound can be naturally occurring or designed in the laboratory. It can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art.

The cell can be any primary human cell or human cell line which expresses a *REG* family gene, or a gene represented by the Hs.111244 polynucleotide, as disclosed above. Methods of establishing cultures of primary human cells or of culturing cell lines are well known in the art.

Expression of at least one gene of the *REG* gene family or the gene represented by the Hs.111244 polynucleotide can be monitored. Expression can be measured in a sample of the same cell population before and after contact with the test compound. Alternatively, control cell populations can be employed. A test compound which decreases expression of at least one member of the *REG* gene family is identified as a potential drug for chronic mucosal injury. A test compound which decreases expression of a gene represented by the Hs.111244 polynucleotide is identified as a potential drug for decreasing ulcerative colitis. Preferably, the test compound decreases the amount of the gene expression product by at least 10%, 25%, 50%, 75% or 100%.

SEQUENCE LISTING

SEQ ID NO:1 cDNA sequence of *pancreatic stone protein (PSP)*

SEQ ID NO:2 cDNA sequence of *pancreatitis-associated protein (PAP)*

SEQ ID NO:3 cDNA sequence of *human pancreatic beta cell growth factor (INGAP)*

SEQ ID NO:4 cDNA sequence of *regenerating gene homologue (REGH)*

SEQ ID NO:5 cDNA sequence of Hs.11124

Example 1

*PSP*, *PAP* and *REGH* are expressed in colonic mucosa of patients with inflammatory bowel disease. Parallel methods of measuring gene expression have been recently developed which allow concurrent measurement of the expression of a large number of genes. Light-directed solid-phase combinatorial chemistry was used to generate oligonucleotide probe arrays which provide representation of nearly 7000 human cDNA and EST sequences. Each gene is represented by 20 individual 25-mer oligonucleotide sequences. mRNA isolated from the mucosa of colonic resection specimens was used to generate hybridization probes for our analysis. Details of the GENECHIP technology, probe synthesis, hybridization, and confocal scanning have been previously described. The fluorescence intensity for different levels of gene expression was standardized by spiking known amounts of control genes into the probe mixture (Figure 1). Detection at 1.5 pM is approximately equal to one message copy per cell. Tissue samples taken from the area used to isolate RNA were sent for histochemistry to be scored for acute and chronic inflammation, ulceration, dysplasia, eosinophilia, epithelial apoptosis, and metaplastic changes. Expression levels of *PSP*, *PAP*, and *REGH* in 15 clinical specimens are shown in Figure 2. *PSP* RNA expression was in the top 2% of all arrayed genes in ulcerative colitis. Expression levels corresponded closely to histologic measures of disease activity. One non-IBD patient with severe acute inflammation, but no ulceration (rectal prolapse-specimen 11), did not express detectable *PSP*, *PAP*, or *REGH*.

Example 2

*Pichia pastoris* expression vectors were constructed with *PSP*, *PAP*, and *REGH*. Gene-specific primer pairs were designed to incorporate a 5' Xho I site, and a portion of the α-factor gene leading up to the yeast Ste13 cleavage site (5' end) and a Xba I containing primer that deleted the

stop codon and included a 3' Myc epitope tag. After Kex2 and Ste13 signal cleavage by *Pichia*, the amino-terminus should be identical to the native secreted protein. RT-PCR was performed using the TITAN One Tube System (Boehringer Mannheim). The PCR products of these reactions, using RNA from a healthy or UC patient are shown in Figure 3. These results demonstrate trace amounts of *PAP* in the "normal" patient examined, but otherwise agree with the results of the GENECHIP hybridization. Bands were gel purified and cloned into pGEM-T (Promega). The Xba/Xho gene fragments were excised and ligated into pPICZ( (Invitrogen). Constructs were bidirectionally sequenced with primers derived from the vector and were found to match the published sequences. Linearized plasmids were transformed into *Pichia* KM71 and recombinant clones identified by Zeocin selection.

Example 3

PSP, PAP, and REGH were expressed in *Pichia pastoris*. PICZα places the inserted gene downstream of a strong methanol-inducible AOX1 promoter. Individual clones were grown in 10 ml cultures of BMGY media overnight and resuspended into 1/5 volume of BMMY (0.5% methanol) for induction. Aliquots of media supernatant were taken at various times after methanol induction and subjected to 15% SDS-PAGE. PSP or REGH expression was identified by the new appearance of 18 and 18.5 kDa bands (respectively), peaking 48 hours after induction. These sizes include the 2.5 kDa C-terminal epitope tag, whose presence was verified by Western blot (ECL) utilizing a monoclonal anti-Myc antibody (Invitrogen). Tryptic digestion of both PSP and REGH led to a mobility reduction of about 1 kDa, reflecting the expected size change following cleavage at the Arg11-Ile12 bond (data not shown). PAP expression was also demonstrated.

Large scale protein purification was performed by directly scaling up the protocol outlined above. *Pichia* from 1.5 liter cultures were resuspended into 300 mls of BMMY induction media and allowed to grow for 48 hours. Culture supernatants were concentrated by ammonium sulfate precipitation and column purified by Bio-Gel P30. Fractions containing PSP or REGH were pooled and concentrated using a Centriprep 10 concentrator (Amicon). Figure 4 shows a Coomassie-stained SDS-PAGE gel of the purified proteins.

Claims:

1. A method of diagnosing chronic inflammatory bowel disease comprising:

detecting at least one gene expression product of the *regenerating (REG)* gene family in a body sample of a first human, wherein the first human is suspected of having chronic inflammatory bowel disease;

identifying the first human as having chronic inflammatory bowel disease if the gene expression product is detected.

2. The method of claim 1 wherein an amount of the gene expression product detected in the body sample of the first human is compared with an amount of the gene expression product detected in a body sample of a second human, wherein the second human is healthy, wherein more of the gene expression product detected in the body sample of the first human than in the body sample of the second healthy human, confirms chronic mucosal injury in the first human.

3. The method of claim 1 wherein the gene expression product of the *REG* gene family is selected from the group consisting of gene expression products of *pancreatic stone protein (PSP), pancreatitis-associated protein (PAP), human pancreatic beta cell growth factor (INGAP),* and *regenerating gene homologue (REGH)* genes.

4. The method of claim 1 wherein the chronic mucosal injury is selected from the

group of diseases consisting of ulcerative colitis and Crohn's disease.

5. The method of claim 1 wherein the body sample is blood.

6. The method of claim 1 wherein the body sample is plasma.

7. The method of claim 1 wherein the body sample is serum.

8. The method of claim 1 wherein the body sample is small intestine or colon tissue.

9. The method of claim 1 wherein the gene expression product is a polypeptide.

10. The method of claim 9 wherein an antibody is used to detect the polypeptide.

11. The method of claim 10 wherein an assay selected from the group consisting of Western blot assay, immunoprecipitation assay, enzyme linked immunoabsorbant assay, quantitative antigen capture-based immunoassay, and radioimmunoassay is used to detect the polypeptide.

12. The method of claim 1 wherein the gene expression product is mRNA.

13. The method of claim 12 wherein an assay selected from the group consisting of

Northern blot assay, DNA array, and ribonuclease protection assay, is used to detect the mRNA.

14. A method to aid in the differentiation of chronic mucosal injury from common acute inflammatory colon disorder and common non-inflammatory benign colon disorder in a human with symptoms of bowel disease comprising:

comparing (a) the amount of at least one gene expression product of the *REG* gene family in a body sample of a first human who is suspected of having bowel disease, with (b) the amount of the gene expression product in a body sample of a second human who is healthy;

identifying the first human as having chronic mucosal injury if the body sample of the first human contains more of the gene expression product than the body sample of the second human.

15. The method of claim 14 wherein the gene expression product of the *REG* gene family is selected from the group consisting of gene expression products of *pancreatic stone protein (PSP), pancreatitis-associated protein (PAP), human pancreatic beta cell growth factor (INGAP),* and *regenerating gene homologue (REGH)* genes.

16. The method of claim 14 wherein the body sample is blood.

17. The method of claim 14 wherein the body sample is plasma.

18. The method of claim 14 wherein the body sample is serum.

19. The method of claim 14 wherein the body sample is small intestine or colon tissue.

20. The method of claim 14 wherein the gene expression product is a polypeptide.

21. The method of claim 20 wherein an antibody is used to quantitate the polypeptide.

22. The method of claim 21 wherein an assay selected from the group consisting of Western blot assay, immunoprecipitation assay, enzyme linked immunoabsorbant assay, quantitative antigen capture-based immunoassay, and radioimmunoassay is used to quantitate the polypeptide.

23. The method of claim 14 wherein the gene expression product is mRNA.

24. The method of claim 23 wherein an assay selected from the group consisting of Northern blot assay, DNA array, and ribonuclease protection assay, is used to detect the mRNA.

25. A method to determine degree of injury to small intestine or colon tissue of a human with chronic mucosal injury comprising the steps of:

determining a quantity of a gene expression product of the *REG* gene family in a body sample of a human having chronic mucosal injury,

correlating the quantity of the gene expression product with the degree of injury to the small intestine or colon.

26. The method of claim 25 wherein the gene expression product of the *REG* gene family is selected from the group consisting of gene expression products of *pancreatic stone protein (PSP), pancreatitis-associated protein (PAP), human pancreatic beta cell growth factor (INGAP),* and *regenerating gene homologue (REGH)* genes.

27. The method of claim 25 wherein the chronic mucosal injury is selected from the group of diseases consisting of ulcerative colitis and Crohn's disease.

28. The method of claim 25 wherein the body sample is blood.

29. The method of claim 25 wherein the body sample is plasma.

30. The method of claim 25 wherein the body sample is serum.

31. The method of claim 25 wherein the body sample is small intestine or colon

tissue.

32. The method of claim 25 wherein the gene expression product is a polypeptide.

33. The method of claim 32 wherein an antibody is used to quantitate the polypeptide.

34. The method of claim 33 wherein an assay selected from the group consisting of Western blot assay, immunoprecipitation assay, enzyme linked immunoabsorbant assay, quantitative antigen capture-based immunoassay, and radioimmunoassay is used to quantitate the polypeptide.

35. The method of claim 25 wherein the gene expression product is mRNA.

36. The method of claim 35 wherein an assay selected from the group consisting of Northern blot assay, DNA array, and ribonuclease protection assay is used to detect the mRNA.

37. A method of monitoring the efficacy of therapy for chronic mucosal injury in a human body sample comprising the steps of:

quantitating at least one gene expression product of the *REG* gene family in a body sample of a human who has been subjected to therapy for chronic mucosal injury;

comparing the quantity of expression product in said sample to the quantity of said gene expression product in a matched body sample of the human at an earlier time, wherein a reduction in the quantity of said gene expression product after therapy is an index of efficacy of the therapy.

38. The method of claim 37 wherein the gene expression product of the *REG* gene family is selected from the group consisting of gene expression products of *pancreatic stone protein (PSP), pancreatitis-associated protein (PAP), human pancreatic beta cell growth factor (INGAP)*, and *regenerating gene homologue (REGH)* genes.

39. The method of claim 37 wherein the chronic mucosal injury is selected from the group of diseases consisting of ulcerative colitis and Crohn's disease.

40. The method of claim 37 wherein the body sample is blood.

41. The method of claim 37 wherein the body sample is plasma.

42. The method of claim 37 wherein the body sample is serum.

43. The method of claim 37 wherein the body sample is small intestine or colon tissue.

44. The method of claim 37 wherein the gene expression product is a polypeptide.

45. The method of claim 44 wherein an antibody is used to quantitate the polypeptide.

46. The method of claim 45 wherein an assay selected from the group consisting of Western blot assay, immunoprecipitation assay, enzyme linked immunoabsorbant assay, quantitative antigen capture-based immunoassay, and radioimmunoassay is used to quantitate the polypeptide.

47. The method of claim 37 wherein the gene expression product is mRNA.

48. The method of claim 47 wherein an assay selected from the group consisting of Northern blot assay, DNA array, and ribonuclease protection assay is used to detect the mRNA.

49. A method of screening compounds for anti-chronic mucosal injury activity comprising:

contacting a colonic cell expressing a gene which is a member of the *REG* gene family with a test compound and;

quantitating expression of the *REG* gene, wherein a test compound which decreases expression of the gene is identified as a potential compound for treating

chronic mucosal injury.

50. The method of claim 49 wherein the gene is selected from the group consisting of *pancreatic stone protein (PSP), pancreatitis-associated protein (PAP), human pancreatic beta cell growth factor (INGAP),* and *regenerating gene homologue (REGH)* genes.

51. A method of diagnosing ulcerative colitis comprising:

detecting an mRNA which is expressed by a gene represented by a Hs.111244 polynucleotide in a body sample of a first human who is suspected of having ulcerative colitis;

identifying the human as having ulcerative colitis if said mRNA is detected.

52. The method of claim 51 wherein an amount of the mRNA detected in the body sample of the first human is compared with an amount of the mRNA in a body sample of a second human who is healthy;

identifying the first human as having ulcerative colitis if the body sample of the first human contains more of the mRNA than the body sample of the second human.

53. The method of claim 51 wherein the body sample is blood.

54. The method of claim 51 wherein the body sample is plasma.

55. The method of claim 51 wherein the body sample is serum.

56. The method of claim 51 wherein the body sample is small intestine or colon tissue.

57. The method of claim 51 wherein an assay selected from the group consisting of Northern blot assay, DNA array, and ribonuclease protection assay is used to detect the mRNA.

58. A method to aid in the differentiation of ulcerative colitis from common acute inflammatory colon disorder, common non-inflammatory benign colon disorder, and Crohn's disease in a human with symptoms of bowel disease comprising:

comparing the amount of mRNA which is expressed by a gene represented by a Hs.111244 polynucleotide in a body sample of a first human suspected of having bowel disease with the amount of the mRNA in a comparable body sample of a second human who is healthy, wherein a body sample of the first human which contains more of the mRNA than the body sample of the second human identifies the first human as having ulcerative colitis.

59. The method of claim 58 wherein the body sample is blood.

60. The method of claim 58 wherein the body sample is plasma.

61. The method of claim 58 wherein the body sample is serum.

62. The method of claim 58 wherein the body sample is small intestine or colon tissue.

63. The method of claim 58 wherein an assay selected from the group consisting of Northern blot assay, DNA array, and ribonuclease protection assay is used to detect the mRNA.

64. A method to determine degree of injury to small intestine or colon tissue of a human with ulcerative colitis comprising the steps of:

determining a quantity of an mRNA which is expressed by a gene represented by a Hs.111244 polynucleotide in a body sample of a first human having ulcerative colitis;

correlating the quantity of the mRNA with the degree of injury to the small intestine or colon.

65. The method of claim 64 wherein the body sample is blood.

66. The method of claim 64 wherein the body sample is plasma.

67. The method of claim 64 wherein the body sample is serum.

68. The method of claim 64 wherein the body sample is small intestine or colon tissue.

69. The method of claim 64 wherein an assay selected from the group consisting of Northern blot assay, DNA array, and ribonuclease protection assay is used to detect the mRNA.

70. A method of monitoring the efficacy of therapy for ulcerative colitis in a human body sample comprising the steps of:

quantitating an mRNA which is expressed by gene represented by a Hs.111244 polynucleotide in a body sample of a human who has been subjected to therapy for ulcerative colitis;

comparing the quantity of the mRNA in said sample to the quantity of said mRNA in a matched body sample of the human at an earlier time, wherein a reduction in the quantity of said mRNA after therapy is an index of efficacy of the therapy.

71. The method of claim 70 wherein the body sample is blood.

72. The method of claim 70 wherein the body sample is plasma.

73. The method of claim 70 wherein the body sample is serum.

74. The method of claim 70 wherein the body sample is small intestine or colon tissue.

75. The method of claim 70 wherein an assay selected from the group consisting of Northern blot assay, DNA array, and ribonuclease protection assay is used to detect the mRNA.

76. A method of screening compounds for anti-ulcerative colitis activity comprising:

contacting a colonic cell expressing an mRNA which is expressed by a gene represented by a Hs.111244 polynucleotide with a test compound and;

quantitating expression of the mRNA by the cell, wherein a test compound which decreases expression of the mRNA is identified as a potential compound for treating ulcerative colitis.

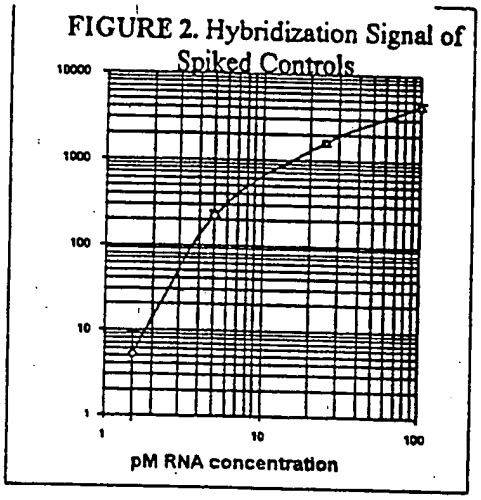


FIG. 1

| Specimen | Ulcerative Colitis | | | | | | | | Normal (non-IBD) | | | | Crohn's disease | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| PSP | 353 | 6549 | 276 | 3695 | 4189 | 2456 | 1008 | 0858 | 0 | 0 | 0 | 311 | 829 | 3104 | 1546 |
| PAP | 146 | 1085 | 130 | 327 | 141 | 1034 | 1031 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 |
| REGH | 0 | 2276 | 0 | 174 | 10 | 87 | 4447 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 128 |

0=Not Detected

FIG. 2

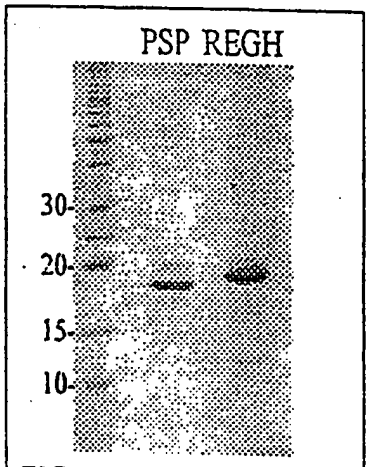


FIGURE 5. Purification of recombinant PSP and REGH

FIG 4

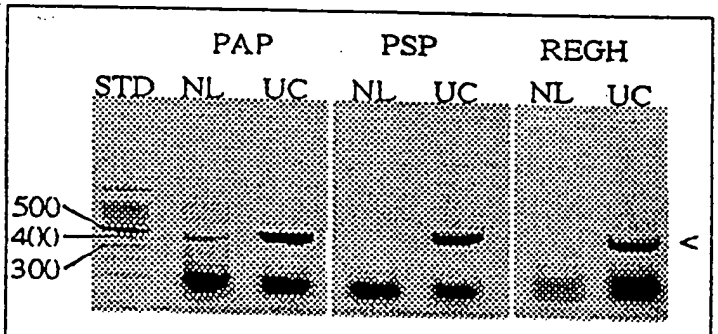


FIGURE 4. Reverse transcriptase-PCR

FIG. 3

SEQUENCE LISTING

<110> Dieckgraefe, Brian K.

<120> Gene Markers for Chronic Mucosal Injury

<130> 04255.75314

<140>

<141>

<160> 5

<170> PatentIn Ver. 2.0

<210> 1

<211> 777

<212> DNA

<213> Homo sapiens

<400> 1

ttcttcaaac cctcctcttc cctgtgttct cctacagaga ttgctgattt ctccttaagc 60

aagagattca ctgccgctaa gcatggctca gaccaactcg ttcttcatgc tgatctcctc 120

cctgatgttc ctgtctctga gccaaggcca agaggcccag acagagttgc cccaggcccg 180

gatcagctgc ccagaaggca ccaatgccta tcgctcctac tgctactact ttaatgaaga 240

ccgtgagacc tgggttgatg cagatctcta ttgccagaac atgaattcgg gcaacctggt 300

gtctgtgctc acccaggccg agggtgcctt tgtggcctca ctgattaagg agagtggcac 360

tgatgacttc aatgtctgga ttggcctcca tgaccccaaa aagaaccgcc gctggcactg 420

gagcagtggg tccctggtct cctacaagtc ctggggcatt ggagccccaa gcagtgttaa 480

tcctggctac tgtgtgagcc tgacctcaag cacaggattc cagaaatgga aggatgtgcc 540

ttgtgaagac aagttctcct ttgtatgcaa gttcaaaaac tagaggcagc tggaaaatac 600

atgtctagaa ctgatccagc aattacaacg gagtcaaaaa ttaaaccgga ccatctctcc 660

aactcaactc aacctggaca ctctcttctc tgctgagctt gcctgttaat tcttccaatag 720

ttttacctac cccagtcttt ggaaccctaa ataataaaaa taaacatgtt ttccact 777

<210> 2

<211> 798

<212> DNA

<213> Homo sapiens

<400> 2

cgggagagtg actcctgatt gcctcctcaa gtcgcagaca ctatgctgcc tcccatggcc 60

ctgcccagtg tatcttggat gctgctttcc tgcctcatgc tgctgtctca ggttcaaggt 120

gaagaacccc agagggaact gccctctgca cggatccgct gtcccaaagg ctccaaggcc 180

tatggctccc actgctatgc cttgtttttg tcaccaaaat cctggacaga tgcagatctg 240

gcctgccaga agcggccctc tggaaacctg gtgtctgtgc tcagtggggc tgagggatcc 300

ttcgtgtcct ccctggtgaa gagcattggt aacagctact catacgtctg gattgggctc 360

catgacccca cacagggcac cgagcccaat ggagaaggtt gggagtggag tagcagtgat 420

gtgatgaatt actttgcatg ggagagaaat ccctccacca tctcaagccc cggccactgt 480

gcgagcctgt cgagaagcac agcatttctg aggtggaaag attataactg taatgtgagg 540

ttaccctatg tctgcaaagt tcactgacta gtgcaggagg gaagtcagca gcctgtgttt 600

ggtgtgcaac tcatcatggg catgagacca gtgtgaggac tcaccctgga agagaatatt 660

cgcttaattc ccccaacctg accacctcat tcttatcttt cttctgtttc ttcctccccg 720

ctagtcattt cagtctcttc attttgtcat acggcctaag gctttaaaga gcaataaaat 780

ttttagtctg caaaaaaa 798

<210> 3

<211> 586

<212> DNA

<213> Homo sapiens

<400> 3

ttcccatgac cctctgtagg atgtcttgga tgctgctttc ctgcctgatg ttcctttctt 60

gggtggaagg tgaagaatct caaaagaaac tgccttcttc acgtataacc tgtcctcaag 120

gctctgtagc ctatgggtcc tattgctatt cactgatttt gataccacag acctggtcta 180

atgcagaact atcctgccag atgcatttct caggacacct ggcatttctt ctcagtactg 240

gtgaaattac cttcgtgtcc tcccttgtga agaacagttt gacggcctac cagtacatct 300

ggattggact ccatgatccc tcacatggta cactacccaa cggaagtgga ctggaagtgga 360

gcagttccaa tgtgctgacc ttctataact gggagaggaa cccctctatt gctgctgacc 420

gtggttattg tgcagttttg tctcagaaat caggttttca gaagtggaga gattttaatt 480

gtgaaaatga gcttccctat atctgcaaat tcaaggtcta gggcagttct aatttcaaca 540

gcttgaaaat attatgaagc tcacatggac aaggaagcaa gtatga 586

<210> 4

<211> 3411

<212> DNA

<213> Homo sapiens

<400> 4

aggaagggca aagctcaaca tcaacttgga cagtttgcca acctgtttgt ggtaagttga 60

tgtcatttgt gaccactcct aatgtgtgcc accaataagc tattcctgat gccagaatct 120

cttactgtca gtgccctctg taggccttct gatccttact ccttgctcca cccattgttt 180

atatcatgta gttctctctc agaccctgat ataaagctcc tactctgtct gacctgacaa 240

gccacctcaa gtggacaagg cacttaccaa caggtaaagg ggcattacag gagaagagca 300

tgtctaacgt gggattttct cttttcattt tgaggtagat acagggtgat tttctgaata 360

aaagatccca gtagtaatga aacttaagca agaccaaagc tgatttcggg taatttggcc 420

tctgttatcc ccaaaccaaa agagaaatat ctgggagtgt agctatctca gtggaccttt 480

ctgctcacag gaattcagag aggagaggat gttagaaaga taacaggtgc tctgctctct 540

tcttcaaacc ctcttccctg tgttctccta cagagattgc tgatttgctc cttaagcaag 600

agattcactg ccgctaagca tggctcagac caactcgttc ttcatgctga tctcctccct 660

gatgttcctg tctctgagcc aaggtgagat tttcccccac acttcccaca accccaactc 720

tgaattcttc cctccatcct catgtataag gttcacttga aaaaaagcag agtcaacatc 780

agggtttttt tatgttgttc agtgatcatt atggctgatt ttatcccatt caaaaacacc 840

ctcaccttca ttcatgggtt tgagacagaa tttaatagga ccacttatag gtgaccattg 900

tggttgagtt tatctgattg aatctatatg cgatggcagt ttgggggatg tttttatgta 960

gtcattgcta ggatgagagc taaggcaaac gtgtgcaggg aaaccgagag aaacttgaga 1020

aaggaggaag cctgggtctt taaaggcaga agcctcagcc tcagaattaa aggaaaacga 1080

gaactcattt atttagccta ttcattgtga gctcttgtct tgagcagagg aaactagaga 1140

gaaaagagat aggatgcagg agggcagaag tgagcaatcg ccccagtatt cactgtatcc 1200

atatgttctt ataaggacac caagaagccc ctattcacct tccagccttt tccttgccct 1260

gagattcttt cttagttatc tccttttttt tttccccagg ccaggagtcc cagacagagc 1320

tgcctaatcc ccgaatcagc tgcccagaag gcaccaatgc ctatcgctcc tactgctact 1380

actttaatga agaccctgag acctgggttg atgcagatgt gagtgaggag agcagcaggg 1440

gaagggaggc ttatgaaggt agaggcagct gctaatttgc agtgtgttct gtggctgcaa 1500

tgagataaga ttgatccctt ccctattcca ccactggtcc aaaacttccc aatctacttt 1560

atcccatcat ttgacacatt cccagcacag agatgctggt ggtcagtgac agcatcatca 1620

gggacatttc tgtgctgtcc tttttctgtt acatcctctg gaaggtctca gtatatccct 1680

cacaccttcc ttctccactg agtgctccat tttcttctcc aacagctcta ttgccagaac 1740

atgaattcag gcaacctggt gtctgtgctc acccaggcgg agggtgcctt cgtggcctca 1800

ctgattaagg agagtagcac tgatgacagc aatgtctgga ttggcctcca tgacccaaaa 1860

aaggtcagtc tgcagccacc tctatctcct tataaacatt tttgagaggt aagagggacg 1920

tttaaggtct ggcaccgcaa tcaccaactt ttatcttttt gtttgtttaa ataaaagcaa 1980

cctctttata gatcctataa tgtatgagtt gtgaagttca gtgtaggtag ttagagacat 2040

gagctgaagg ctgaattttc tgggctctgg gaattcatgc acccactcat tgtgtctact 2100

tctagaaatg catctttatg tacaactttt tccctatttt gctattgtct gtcttggaag 2160

aggtccctct gtagactata tagaaaatga gtagtggagg agaatctact gctggcattt 2220

gttatacatt ttatacaagt gtataaaact gtacagtata ttatttagtt taacactata 2280

aactaaataa tatatcaaca actactctac agccaatgtt atgctggata tgagagttct 2340

gagattcagg aaaaaaatca gaaaccactc tctgtaatgg gcttttatgg gtctctgtat 2400

caaattctga acacttatta tttgctagaa gaggaggagg aattcggaca ttctagagaa 2460

ggagaagctt agagcaaaag cagaggaaat gatatgatat tcatggtgac aacaatgttt 2520

attctttctg ctataacttg gcctgtttct gagtgtgcac acaggcctgg ttattctatt 2580

gatttttgag tgaccatggc ccctgttctg gcccttctcc atctagaacc gccgctggca 2640

ctggagtagt gggtccctgg tctcctacaa gtcctgggac actggatccc cgagcagtgc 2700

taatgctggc tactgtgcaa gcctgacttc atgctcaggt gagaggcaga caatctatcc 2760

acctgttgcc atttccttcc cacttatctc tggggatgaa catggggact gggatagagg 2820

aaaggtaagc tccttatctg gaaaataaag aagtatttcc tctagttttt tgttctgagt 2880

cctaggttga ggaggggcta cactccttct gatcctctat gtctgacact tctcattgta 2940

ctataggatt caagaaatgg aaggatgaat cttgtgagaa gaagttctcc tttgtttgca 3000

agttcaaaaa ctagaggaag ctgaaaaatg gatgtctaga actggtcctg caattactat 3060

gaagtcaaaa attaaactag actatgtctc caactcagtt cagaccatct cctccctaat 3120

gagtttgcat cgctgatctt cagtaccttc acctgtctca gtctctagag ccctgaaaaa 3180

taaaaacaaa cttattttta tccagtgttc tgtcttctgc atttgctctt tctacagccc 3240

atgcttgggt ggttggggtg ggaatgattg tcacactcca gagcttgcca tggcccatcc 3300

acttgttaaa accccactca cattttatgt atgtcaggct tatgaacatg tggtggcctt 3360

gtttatgaca agataaaaag attaagattt catccacaac acatgttagc a 3411

<210> 5

<211> 1734

<212> DNA

<213> Homo sapiens

<400> 5

gcgagcgtgg acctgggacg ggtctgggcg gctctcggtg gttggcacgg gttcgcacac 60

ccattcaagc ggcaggacgc acttgtctta gcagttctcg ctgaccgcgc tagctgcggc 120

ttctacgctc cggcactctg agttcatcag caaacgccct ggcgtctgtc ctcaccatgc 180

ctagcctttg ggaccgcttc tcgtcgtcgt ccacctcctc ttcgccctcg tccttgcccc 240

gaactcccac cccagatcgg ccgccgcgct cagcctgggg gtcggcgacc cgggaggagg 300

ggtttgaccg ctccacgagc ctggagagct cggactgcga gtccctggac agcagcaaca 360

gtggcttcgg gccggaggaa gacacggctt acctggatgg ggtgtcgttg cccgacttcg 420

agctgctcag tgaccctgag gatgaacact tgtgtgccaa cctgatgcag ctgctgcagg 480

agagcctggc ccaggcgcgg ctgggctctc gacgccctgc gcgcctgctg atgcctagcc 540

agttggtaag ccaggtgggc aaagaactac tgcgcctggc ctacagcgag ccgtgcggcc 600

tgcgggggge gctgctggac gtctgcgtgg agcagggcaa gagctgccac agcgtgggcc 660

agctggcact cgaccccagc ctggtgccca ccttccagct gaccctcgtg ctgcgcctgg 720

actcacgact ctggcccaag atccaggggc tgtttagctc cgccaactct cccttcctcc 780

ctggcttcag ccagtccctg acgctgagca ctggcttccg agtcatcaag aagaagctgt 840

acagctcgga acagctgctc attgaggagt gttgaacttc aacctgaggg ggccgacagt 900

gccctccaag acagagacga ctgaactttt ggggtggaga ctagaggcag gagctgaggg 960

actgattcct gtggttggaa aactgaggca gccacctaag gtggaggtgg gggaatagtg 1020

tttcccagga agctcattga gttgtgtgcg ggtggctgtg cattggggac acataccct 1080

cagtactgta gcatgaaaca aaggcttagg ggccaacaag gcttccagct ggatgtgtgt 1140

gtagcatgta ccttattatt tttgttactg acagttaaca gtggtgtgac atccagagag 1200

cagctgggct gctcccgccc cagcccggcc cagggtgaag gaagaggcac gtgctcctca 1260

gagcagccgg agggaggggg gaggtcggag gtcgtggagg tggtttgtgt atcttactgg 1320

tctgaaggga ccaagtgtgt ttgttgtttg ttttgtatct tgtttttctg atcggagcat 1380

cactactgac ctgttgtagg cagctatctt acagacgcat gaatgtaaga gtaggaaggg 1440

gtgggtgtca gggatcactt gggatctttg acacttgaaa aattacacct ggcagctgcg 1500

tttaagcctt cccccatcgt gtactgcaga gttgagctgg caggggaggg gctgagaggg 1560

tgggggctgg aacccctccc cgggaggagt gccatctggg tcttccatct agaactgttt 1620

acatgaagat aagatactca ctgttcatga atacacttga tgttcaagta ttaagaccta 1680

tgcaatattt tttacttttc taataaacat gtttgttaaa acaaaaaaaa aaaa 1734

**PCT** WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/68, G01N 33/68 — A3

(11) International Publication Number: WO 00/14283

(43) International Publication Date: 16 March 2000 (16.03.00)

(21) International Application Number: PCT/US99/20098

(22) International Filing Date: 3 September 1999 (03.09.99)

(30) Priority Data:
09/146,969 4 September 1998 (04.09.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/146,969 (CON)
Filed on 4 September 1998 (04.09.98)

(71) Applicant *(for all designated States except US)*: WASHINGTON UNIVERSITY [US/US]; 660 South Euclid Avenue, Campus Box 8013, St. Louis, MO 63110 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: DIECKGRAEFE, Brian, K. [US/US]; Washington University School of Medicine, Department of Gastroenterology, Box 8124, 660 South Euclid Avenue, St. Louis, MO 63110 (US).

(74) Agents: KAGAN, Sarah, A. et al.; Banner & Witcoff, Ltd., 11th floor, 1001 G Street, N.W., Washington, DC 20001–4597 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**
*With international search report.*

(88) Date of publication of the international search report:
2 June 2000 (02.06.00)

(54) Title: GENE MARKERS FOR CHRONIC MUCOSAL INJURY

(57) Abstract

The invention provides gene markers for chronic mucosal injury and ulcerative colitis. Expression products of the *REG* gene family can be used to detect the presence of chronic mucosal injury in a body sample of a human. The expression products of a gene represented by a Hs.111244 polynucleotide can be used to detect ulcerative colitis in a body sample of a human. Further, these markers can be used to differentiate humans with chronic mucosal injury from humans with common acute inflammatory colon disorder, common non–inflammatory benign colon disorder, and healthy colons. The degree of injury to the colon from chronic mucosal injury can be determined and the efficacy of therapy for chronic mucosal injury can be monitored. A method of screening compounds for anti–chronic mucosal injury and anti–ulcerative activity is also provided by these gene markers.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | | | TR | Turkey |
| BG | Bulgaria | HU | Hungary | ML | Mali | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MN | Mongolia | UA | Ukraine |
| BR | Brazil | IL | Israel | MR | Mauritania | UG | Uganda |
| BY | Belarus | IS | Iceland | MW | Malawi | US | United States of America |
| CA | Canada | IT | Italy | MX | Mexico | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NE | Niger | VN | Viet Nam |
| CG | Congo | KE | Kenya | NL | Netherlands | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NO | Norway | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | NZ | New Zealand | | |
| CM | Cameroon | | | PL | Poland | | |
| CN | China | KR | Republic of Korea | PT | Portugal | | |
| CU | Cuba | KZ | Kazakstan | RO | Romania | | |
| CZ | Czech Republic | LC | Saint Lucia | RU | Russian Federation | | |
| DE | Germany | LI | Liechtenstein | SD | Sudan | | |
| DK | Denmark | LK | Sri Lanka | SE | Sweden | | |
| EE | Estonia | LR | Liberia | SG | Singapore | | |